

## Palynological Investigation of Some *Trifolium* L. (Fabaceae) Species Distributed in Şanlıurfa

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**Abstract:** In this study, the pollen morphology of 5 species (*Trifolium boissieri* Guss. ex-Soy. -Will. & Godr., *T. dasycarpum* C.Presl, *T. pauciflorum* d'Urv., *T. scabrum* L., and *T. spumosum* L.) belonging to the genus *Trifolium* L. from the Fabaceae family, distributed in Şanlıurfa province, was examined with light and electron microscopes. Within the framework of the palynological study, pollen shapes, ornamentations, pore and colpus lengths and widths were determined and their measurements were given. Pollen grains are generally monad in structure, have trizonocolporate aperture, and subprolate to prolate-spheroidal shape. Ornamentation generally shows significant differences in polar and equatorial regions. In the pollen grains examined, perforate, psilate-perforate, and reticulate ornamentation is seen in the polar region, while microreticulate and reticulate ornamentation is dominant in the equatorial region. While the polar axis lengths were determined to be between 38.67-29.19 µm on average, the equatorial axis length was determined to be between 33.79-23.45 µm on average. As a result of the study, it was determined that characters such as pollen shape, pollen size, and surface ornamentation differed among species and these were the characters that could have taxonomic value in the systematic distinction of species.

**Keywords:** Pollen, morphology, palynology, Türkiye.

### Şanlıurfa'da Yayılış Gösteren Bazı *Trifolium* L. (Fabaceae) Türlerinin Palinolojik Yönden İncelenmesi

**Öz:** Bu çalışmada, Şanlıurfa ilinde yayılış gösteren Fabaceae familyasından *Trifolium* L. cinsine ait 5 türün (*Trifolium boissieri* Guss. ex Soy.-Will. & Godr., *T. dasycarpum* C.Presl, *T. pauciflorum* d'Urv., *T. scabrum* L., *T. spumosum* L.) polen morfolojisi ışık ve elektron mikroskopları ile incelenmiştir. Palinolojik çalışma çerçevesinde polen şekilleri, polen yüzey ornamentasyonları, por ve kolpus uzunlukları ve genişlikleri tespit edilmiş ve ölçümleri verilmiştir. Polenler genel monad yapıda, trizonokolporat apertüre sahip ve subprolat ile prolat-sferoidal şekle sahiptir. Ornamentasyon ise genellikle polar ve ekvatorial bölgede belirgin farklılıklar göstermektedir. İncelenen polenlerde polar bölgede perforat, psilat-perforat ve retikalat ornamentasyon görülürken Ekvatorial bölgede ise mikoretikül ve retikül ornamentasyon baskındır. Polar eksen uzunlukları ortalama 38,67- 29,19 µm arasında tespit edilirken ekvatorial eksen uzunluğu ise ortalama 33,79- 23,45 µm arasında tespit edilmiştir. Polen şekli, polen büyüklüğü ve yüzey ornamentasyon gibi karakterlerin türler arasında farklılık gösterdiği ve türlerin sistematik ayrımında taksonomik değere sahip karakterler olduğu tespit edilmiştir.

**Anahtar kelimeler:** Polen, morfoloji, palinoloji, Türkiye.

#### 1. Introduction

The flora of Türkiye is among the world's leading flora in terms of species richness. There are 167 families, 1320 genera, and a total of 11707 taxa belonging to these genera in Turkey. The Fabaceae family is the world's third-largest family of seed plants, with 770 genera and approximately 20,000 species. Approximately 1228 taxa belonging to 72 genera are distributed in Türkiye, making it the country's second-largest family in species number. The number of endemic Fabaceae taxa in Türkiye is 383 (Güner et al., 2012; Christenhusz & Byng, 2017).

*Trifolium* L. (Fabaceae), which exhibits the highest species richness in the Mediterranean basin, is represented by approximately 300 species in the world, while in Türkiye, it is represented by 107 species and 167 taxa. Fourteen of these taxa are determined to be endemic to Türkiye. The genus *Trifolium* is divided into seven sections (Güner et al., 2012; Keskin et al., 2023).

The genus is known by local names such as "Üçgül" or "Yonca" among the public. Since the genus *Trifolium* is also important as meadow plants for fattening and dairy animals, it is one of the economically valuable genera in the Fabaceae family (Güner et al., 2012; Keskin et al., 2023).

The genus *Trifolium* is not easy to classify in classical sense due to the richness of its species. For this reason, important characteristics such as pollen, chromosomes, and genetic characteristics are frequently used in genus systematics (Keskin, 2001a, 2001b; Güner et al., 2012). Palynological and seed morphology studies are frequently used in the classification of the Fabaceae family (Pınar et al., 2009; Çeter et al., 2012; İşgör et al., 2012; Çeter et al., 2013a; 2013b; Kahraman et al., 2013; Pınar et al., 2014; Karaman et al., 2017; Metin et al., 2018; Khan et al., 2019; Altın et al., 2021; Bapir & Galalaey, 2023; Liao et al., 2021; Taşlıyurt et al., 2023; Altın et al., 2024). Pollen grains of taxa belonging to the genus *Trifolium* also show variations among species. *Trifolium* pollen grains generally have different shapes,

from prolate to spheroidal. Apertures can be of different types such as tricolporate, tricolpate or polycolpate. Ornamentation and pollen size also show significant variations among species (Gazar, 2003; Taia, 2004; Koçyiğit et al., 2013; Öztürk, 2013; Akçin et al., 2017).

In this study, a palynological study was carried out on some species of the genus *Trifolium* distributed in Şanlıurfa. Pollens belonging to the species were examined under light microscope (LM) and scanning electron microscope (SEM) to determine pollen morphology.

## 2. Material and Method

The study material consists of *Trifolium boissieri*, *T. dasyurum*, *T. scabrium*, *T. spumosum*, and *T. pauciflorum* species distributed in Şanlıurfa (Table 1). The collected species were identified with the help of the work called Flora of Turkey (Davis, 1965-1988; Davis et al., 1988). The samples were kept at Harran University Herbarium (HARRAN).

### 2.1. Palynological Method

#### 2.1.1. Light Microscope Method

Pollen morphology studies using light microscopy were performed according to the Wodehouse (1935) method. Pollen samples were examined and photographed using a light microscope equipped with a Leica DM3000 Digital Imaging System using 100x immersion objective. For each analyzed character, measurements were made from 20 pollen samples and the average was taken. Pollen equatorial (E) and polar axis lengths (P), exine and intine thicknesses, colpus length (Clg) and width (Clt), and pore length (Plg) and width (Plt) were measured. Measurements were performed using the AlaMet, S.0.06 program.

#### 2.1.2. Scanning electron microscope (SEM) method

Pollen samples were placed on aluminum staples using double-sided adhesive tape. Pollen samples were coated with gold using a Cressington Sputter Coater device, and then microphotographs were taken using a Quanta FEG 250 model Scanning Electron Microscope (SEM). Pollen surface morphology and ornamentation analysis of taxa were performed using SEM microphotographs and relevant literature (Ertzman, 1969; Faegri & Iversen, 1975; Punt et al., 2007; Hesse et al., 2009).

The morphological features of the pollen grains were determined by taking into account pollen shape, polar axis, equatorial axis, exine and intine thickness, ornamentation, colpus length, colpus width, and colpus ornamentation characters.

## 3. Results

The pollen grains examined were generally found to have monad, trizonocolporate aperture structure, and subprolate and prolate-spheroidal shapes. Ornamentation generally showed significant differences in the polar and equatorial areas. Perforate, psilate-perforate, and reticulate ornamentation were seen in the polar area, while microreticulate and reticulate ornamentation were dominant in the equatorial area. While the polar axis lengths were determined to be between 38.67 and 29.19 on average, the equatorial axis length was determined to be between 33.79 and 23.45 on average (Table 2).

### 3.1. *Trifolium boissieri*

Pollen grains were radially symmetric, isopolar, and trizonocolporate. The polar axis was 29.19  $\mu\text{m}$  and the equatorial axis was 23.45  $\mu\text{m}$ . The P/E ratio was calculated as 1.26. According to this ratio, the pollen shape was subprolate. In polar appearance, it was circular. Ornamentation was perforated in the polar area and around the aperture and microreticulate in the equatorial area. Exine thickness was 0.77  $\mu\text{m}$ , and intine thickness was 0.35  $\mu\text{m}$ . Colpus is thin, long (Clg 17.41  $\mu\text{m}$ , Clt 2.5  $\mu\text{m}$ ) and operculate. The operculum membrane had psilate ornamentation. The pore was longitudinal, Plg was 6  $\mu\text{m}$ , and Plt was 4.83  $\mu\text{m}$  (Fig. 1).

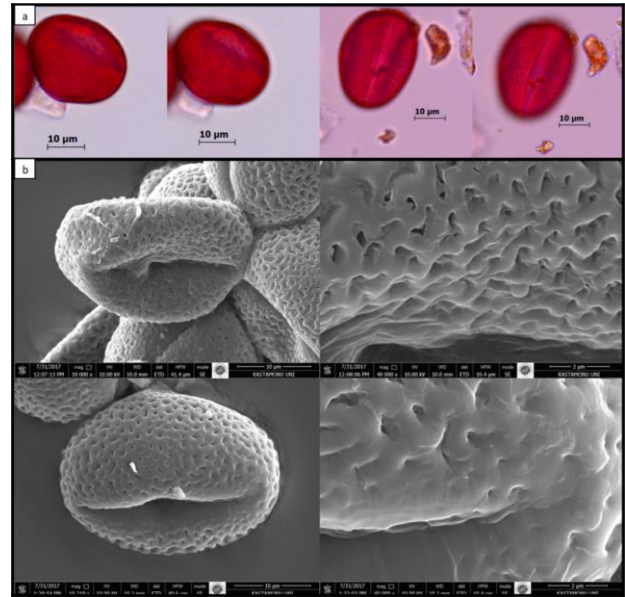


Figure 1. a. Light microscope photographs of *T. boissieri*, b. SEM photographs

### 3.2. *Trifolium dasyurum*

Pollen grains were radially symmetric, isopolar, and trizonocolporate. The polar axis was 38.67  $\mu\text{m}$ , and the equatorial axis was 33.79  $\mu\text{m}$ . The P/E ratio was calculated as 1.07. According to this ratio, the pollen shape was determined as prolate-spheroidal. In the polar view, it was circular-semi triangular. Ornamentation was determined as psilate around the aperture and reticulate in polar and equatorial areas. Exine thickness was determined as 0.87  $\mu\text{m}$  and intine thickness was determined as 0.53  $\mu\text{m}$ . Colpus was thin, long (Clg 28.41  $\mu\text{m}$ , Clt 4.5  $\mu\text{m}$ ) and operculate. Operculum membrane had psilate ornamentation. Pores were longitudinally extended and had a suboblate shape. Plg was determined as 12.41  $\mu\text{m}$  and Plt as 11.58  $\mu\text{m}$  (Fig. 2).

### 3.3. *Trifolium scabrum*

Pollen grains were radially symmetric, isopolar, and trizonocolporate. The polar axis was 38.45  $\mu\text{m}$  and the equatorial axis was 29.10  $\mu\text{m}$ . The P/E ratio was calculated as 1.32. According to this ratio, the pollen shape was subprolate. In polar view, it was semi-triangular. Ornamentation was determined as psilate-perforate around the aperture, and as microreticulate in the polar region and equatorial region. Exine thickness was 0.78  $\mu\text{m}$ , and intine thickness was 0.43  $\mu\text{m}$ . Colpus was thin, long (Clg 25.09  $\mu\text{m}$ , Clt 2.48  $\mu\text{m}$ ), and operculate. Operculum

membrane had granulate ornamentation. The pore was longitudinally extended and had a suboblate shape. Plg was determined as 5.99  $\mu\text{m}$  and Plt as 7.47  $\mu\text{m}$  (Fig. 3).

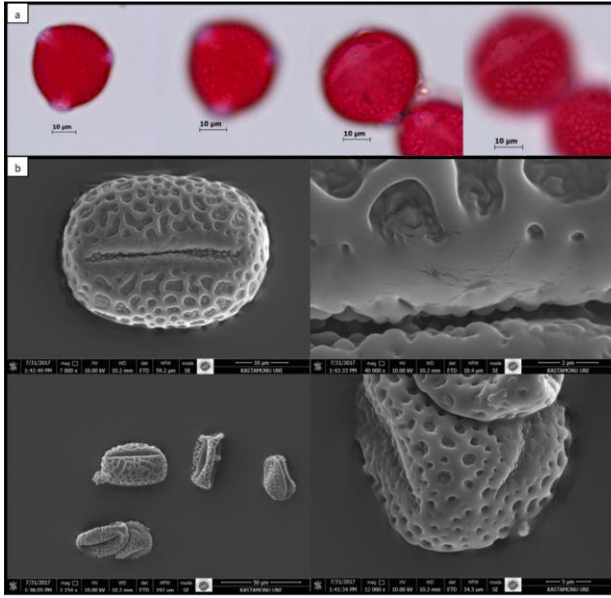


Figure 2. a. Light microscope photographs of *T. dasyurum*, b. SEM photographs

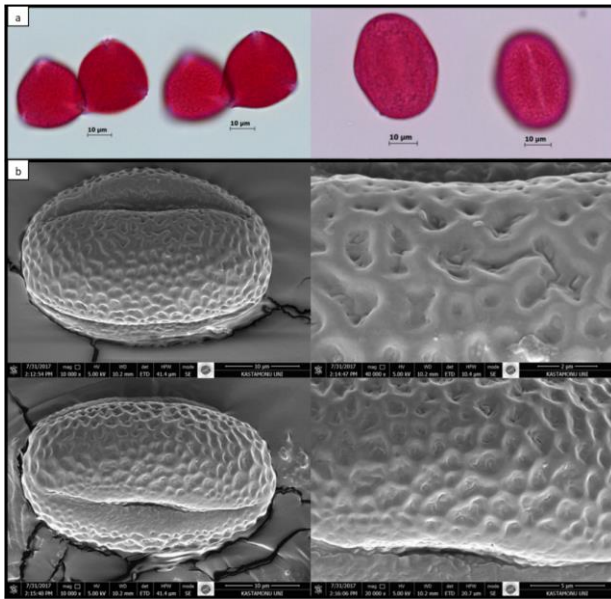


Figure 3. a. Light microscope photographs of *T. scabrum*, b. SEM photographs

### 3.4. *Trifolium spumosum*

Pollen grains were radially symmetric, isopolar, and trizonocolporate. The polar axis was 33.25  $\mu\text{m}$  and the equatorial axis was 27.05  $\mu\text{m}$ . The P/E ratio was calculated as 1.22. According to this ratio, the pollen shape was subprolate. In polar view, it was circular in shape. Ornamentation was determined as psilate-perforate around the aperture and as reticulate in the polar and equatorial area. Exine thickness was 0.72  $\mu\text{m}$  and intine thickness was 0.41  $\mu\text{m}$ . Colpus was thin, long (Clg 26.43  $\mu\text{m}$ , Clt 3.10  $\mu\text{m}$ ), and operculate. The operculum membrane had granulate ornamentation. The pore was longitudinally extended and had a prolate-spheroidal shape. Plg was determined as 8.28  $\mu\text{m}$  and Plt as 7.53  $\mu\text{m}$

(Fig. 4).

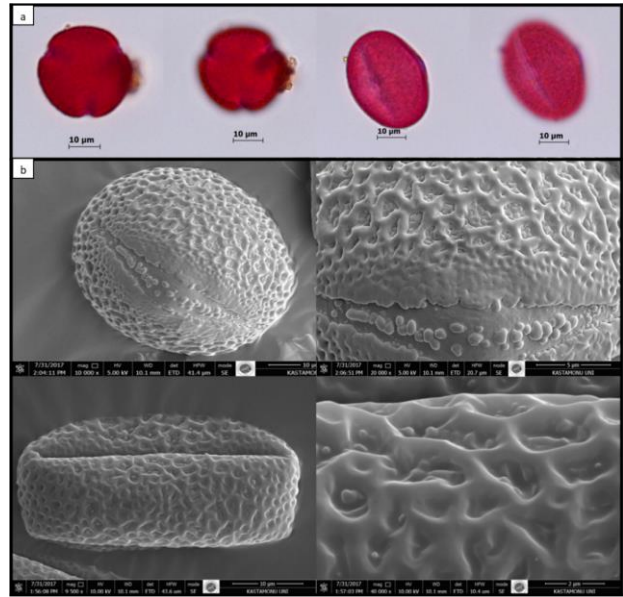


Figure 4. a. Light microscope photographs of *T. spumosum*, b. SEM photographs

### 3.5. *Trifolium pauciflorum*

Pollen grains were radially symmetric, isopolar, and trizonocolporate. The polar axis was determined as 33.84  $\mu\text{m}$  and the equatorial axis as 29.74  $\mu\text{m}$ . The P/E ratio was calculated as 1.13. According to this ratio, the pollen shape was determined as prolate spheroidal. In polar appearance, it was circular. Ornamentation was determined as perforate around aperture and microreticulate in polar equatorial area. Exine thickness was determined as 0.53  $\mu\text{m}$  and intine thickness was determined as 0.28  $\mu\text{m}$ . Colpus was thin, long (Clg 25.8  $\mu\text{m}$ , Clt 3.22  $\mu\text{m}$ ), and operculate. Operculum membrane had granulate ornamentation. Pore was longitudinally extended and had subprolate shape. Plg was determined as 11.75  $\mu\text{m}$  and Plt was determined as 9.52  $\mu\text{m}$  (Fig. 5).

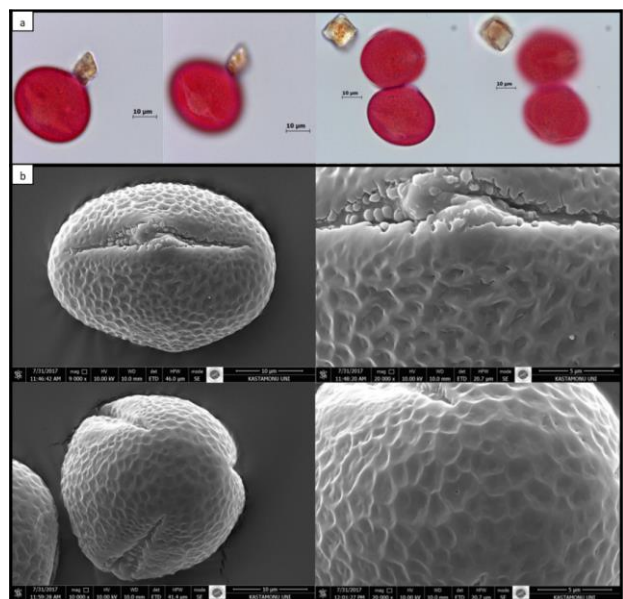


Figure 5. a. Light microscope photographs of *T. pauciflorum*, b. SEM photographs

Table 1. Locality where the samples were collected

Species	Locality	Flowering Period
<i>T. boissieri</i>	Şanlıurfa-Hilvan 10th km, Karaköprü location, 550 m, roadside	April-May
<i>T. spumosum</i>	Şanlıurfa-Bozova road: Around Kızlar village, 720 m, field edge, Şanlıurfa-Hilvan highway 10th km, Karaköprü location, 550 m, roadside, Şanlıurfa-Hilvan highway 32nd km, opposite the airport, 700 m, roadside	April-May
<i>T. scabrum</i>	Şanlıurfa-Hilvan highway 10th km, Karaköprü location,	April-May
<i>T. dasyurum</i>	Şanlıurfa-Viranşehir karayolu 45. km, 665 m, step-kayalık, Şanlıurfa-Viranşehir, step-kayalık, Şanlıurfa - Viranşehir 43. km,	April-May
<i>T. pauciflorum</i>	Şanlıurfa: Center, Osmanbey campus, 500 m, step, Şanlıurfa-Bozova road: Tektaş village junction, 720 m, field edge, Şanlıurfa-Hilvan highway 10 km, Karaköprü location, Şanlıurfa-Viranşehir 45 km, 665 m, steppe-rocky	April-May

Table 2. Pollen characteristics of *Trifolium* taxa

Taxa	Polar axis (P) (µm)			Equatorial axis (E) (µm)			P / E ratio and Pollen shape	Aperture type	Ornamentation		Colpus		Porus		Exine	Intine
	Min.	Mean	Max.	Min.	Mean	Max.			Polar area and Apertur surroundings	Equatorial area	Clg	Clt	Plg	Plt		
1 <i>T. boissieri</i>	25.41	29.19	34.75	20.83	23.45	25.25	1.26/ subprolate	Trizonocolporate	Perforate	Microreticulate	17.41	2.5	6.0	4.83	0.77	0.35
2 <i>T. dasyurum</i>	26.91	38.67	48.91	21.41	33.79	37.0	1.07/ prolate-Spheroidal	Trizonocolporate	Reticulate	Reticulate	28.41	4.5	12.41	11.58	0.87	0.53
3 <i>T. scabium</i>	35.61	38.45	41.14	28.38	29.10	30.28	1.32/ subprolate	Trizonocolporate	Perforat. Psilate-Perforate	Microreticulate	25.09	2.48	5.99	7.47	0.78	0.43
4 <i>T. spumosum</i>	25.77	33.25	38.19	25.42	27.08	29.42	1.22/ subprolate	Trizonocolporate	Perforate. Psilate-Perforate	Reticulate	26.43	3.10	8.28	7.53	0.72	0.41
5 <i>T. pauciflorum</i>	32.44	33.84	34.55	28.66	29.74	30.88	1.13/ prolate-Spheroidal	Trizonocolporate	Perforate	Microreticulate	25.8	3.22	11.75	9.52	0.53	0.28

\* Clg: Colpus width, Plg: Pore width, \* Clt: Colpus length, Plt: Pore length

It is seen that the examined *Trifolium* taxa have significant similarities and differences when their pollen morphologies are mixed. Pollen shape, aperture structure, and ornamentation types are significantly different among the taxa. Other studies examining the pollen morphology of the genus *Trifolium* also show that these characters have important taxonomic diversity.

Koçyiğit et al. (2013) studied the pollen morphology of 16 *Trifolium* taxa collected from Istanbul province. Pollen shapes in the examined taxa were determined as prolate, subprolate, and spheroidal. Ornamentation was determined as reticulate and scabrate. Polar axis lengths were determined between 37.83 µm- 20.41 µm, while equatorial axis lengths were determined between 41.25 µm- 22.10 µm.

Gazar (2003) grouped *Trifolium* taxa collected in Egypt into two subclasses according to pollen morphology. The pollen of the taxa in the first group was circular and triangular in polar view and had a trizonocolporate aperture structure, while the pollen of the second group was semicircular-circular and semitriangular in polar view and had a tricolpate aperture structure.

Another study conducted in Egypt examined the pollen morphology of 12 *Trifolium* taxa. Similarly, it was determined that the pollen had prolate-spheroidal, subprolate, and perprolate shapes. Aperture types were determined to be in different structures as tricolporate, tricolpate, and polycolpate (Taia, 2004).

#### 4. Discussion and Conclusions

In this study, the palynologically compared different aspects of *T. scabrum*, *T. boissieri*, *T. dasyurum*, *T. pauciflorum*, and *T. spumosum* species were determined and tried to be clarified. Significant differences were determined among the pollen types of the examined taxa in terms of size, shape, ornamentation, and aperture measurements. However, all of the examined pollen grains of *Trifolium* taxa were determined to be radially symmetric, isopolar, and trizonocolporate

As a result of the study, similarities and differences of the species that are difficult to distinguish from each other morphologically were revealed by examining their palynological features. Systematics using only morphological characters can create many taxonomic difficulties. Palynological analyses can be a source for systematic studies to overcome these difficulties.

In this study, pollen morphologies of taxa were examined by light microscopy and scanning electron microscopy (SEM). In the literature review, no comprehensive studies were found to determine the pollen morphologies of *T. scabrum*, *T. boissieri*, *T. dasyurum*, *T. pauciflorum*, and *T. spumosum* species. For this reason, this study will guide and enlighten future studies.

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**Ethics committee approval:** Ethics committee approval is not required for this study

**Conflict of interest:** The authors declare that there is no conflict of interest.

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